Tobacco Etch Virus Protease (TEV protease)

Technical Manual No. TM0643 **Version** 07262016

Product Name	Cat.No.	Size
Tobacco Etch Virus Protease (TEV protease)	Z03030-1K	1000 IU
	Z03030-10K	10000 IU

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I. Description

The TEV protease is a highly site-specific cysteine protease that is found in the Tobacco Etch Virus (TEV). The optimum recognition site for this enzyme is the sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser) (ENLYFQ(G/S)) and cleavage occurs between the Gln and Gly/Ser residues, The most commonly used sequence is ENLYFQG. The protease is used to cleave affinity tags from fusion proteins. The optimal temperature for cleavage is 30°C; also it can be used at temperatures as low as 4°C. It is recommended that the cleavage for each fusion protein be optimized by varying the amount of Recombinant Viral TEV Protease, reaction time, or incubation temperature. It can be removed by Ni^{2+} affinity resin.

II. Component

- 1,000 IU(or 10,000 IU) Recombinant Tobacco Etch Virus Protease (TEV) (in 50 mM Tris, 5 mM DTT, 50% glycerol, pH7.5.)

Storage: Store at -20°C.

III. Dialysis of the Fusion Protein

If the buffer of fusion protein contains the following reagents, it is necessary to dialyze your fusion protein before the digestion.

1. > 2 M urea, >0.5 M Guanidine hydrochloride or > 50 mM imidazole.
2. pH values below 6 and above 9.
3. Avoid the presence of cysteine protease inhibitors.

IV. TEV Digestion

One unit of TEV protease cleaves >85% of 3 μg of control substrate in 1 hour at pH 8.0 at 30°C. However, because each target protein has the different position of cleavage site, it is recommended to optimize the concentrations of TEV, incubation time and temperatures in order to find the best cleavage condition.

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Small scale optimization

For the small cleavage, TEV can be diluted using TEV / Storage Buffer. The following is an example of optimizing the cleavage condition.

1. 10X TEV Cleavage/Capture Buffer (200 mM Tris-HCl, 500 mM NaCl, pH 7.4)
2. Set up 2 reactions, including a reaction without TEV for the control:

Fusion Protein	18 µg
10X TEV Cleavage/Capture Buffer	5 µl
TEV (add 1 µl TEV Dilution/Storage Buffer for the control)	6 IU
Deionized Water	x µl
Total volume	50 µl

3. Mix well and incubated at 30°C of 1h, 3h or 4°C overnight (~16 hours).
4. Load 10 µl on an SDS-PAGE gel to determine the best cleavage result. If the result is unsuitable, the optimization of temperatures can be tested.

Scale up

Scale up the reaction proportionality according to the best cleavage result.

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